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Tyrosine Depletion Alters Cortical and Limbic Blood Flow but Does Not Modulate Spatial Working Memory Performance or Task-Related Blood Flow in Humans

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Abstract: Dopamine appears critical in regulating spatial working memory (SWM) within the PFC of non-human primates; however findings in humans are less clear. Recent studies of the effects of global depletion of dopamine via acute tyrosine/phenylalanine depletion (TPD) on SWM task performance have yielded inconsistent results, which may be partly related to task differences. These previous studies do not address whether TPD can directly impair PFC functioning. The current study investigated the effects of TPD on (1) regional cerebral blood flow (rCBF) during a SWM n-back task using $H_2{}^{15}O$ Positron Emission Tomography (PET), and (2) behavioural performance on three different SWM tasks. Ten healthy males were scanned twice: once following a placebo (balanced) amino acid mixture and once following an equivalent mixture deficient in tyrosine/phenylalanine (TPD condition). Participants completed two additional delayed-response tasks to examine whether differences in response demands influenced TPD effects on performance. TPD resulted in widespread increases in rCBF, with maximum increases in the region of the parahippocampal gyrus bilaterally, left inferior frontal gyrus, and the putamen. TPD related rCBF reductions were observed in the medial frontal gyrus bilaterally, right inferior temporal gyrus and the pons. Despite widespread changes in blood flow following TPD, no specific effects on SWM neural networks or task performance were observed. The use of three different SWM tasks suggests that task differences are unlikely to account for the lack of effects observed. These findings question the capacity of TPD to consistently modulate dopamine function and SWM neural networks in humans. *Hum Brain Mapp 28:1136–1149, 2007.*

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INTRODUCTION

Dopamine (DA) appears to play a regulatory role in working memory function within the prefrontal cortex (PFC) of the non-human primate, with the two major subtypes of receptor (DA D_1 and DA D_2) proposed to differentially modulate behaviour. DA D_1 receptor antagonists appear to modulate "memory fields" within the PFC during the delay period of a spatial working memory (SWM) task [for review, see Goldman-Rakic et al., 1996], with the DA D_2 receptor more involved in response preparation within the PFC [Wang et al., 2004]. Prefrontal D_1 receptor availability and abnormal activity of the PFC have both been linked to deficits in working memory in schizophrenia [Abi-Dargham et al., 2002]; however similar relationships have not been clearly demonstrated in healthy volunteers and the nature of the relationship between DA, working memory and prefrontal cortical functioning in humans remains poorly understood.

This may be due in part to the lack of appropriate pharmacological tools for selectively probing the D_1 receptor in humans. To date, research in humans has focused on agonists/antagonists of the D_2 receptor, or combined D_1/D_2 agonists and antagonists [for a review, see Ellis and Nathan, 2001]. It has been well documented that studies with D_2 agonists/antagonists have yielded inconsistent results, with some studies observing changes in working memory performance [Luciana and Collins, 1997; Luciana et al., 1992; Mehta et al., 2001], and other studies observing no effect [Bartholomeusz et al., 2003; Kimberg et al., 1997; Muller et al., 1998]. The effects of the combined D_1/D_2 receptor agonist pergolide have also been inconsistent, with evidence for enhancing working memory performance [Muller et al., 1998], improving performance in only some individuals, dependent on working memory capacity [Kimberg and D'Esposito, 2003], or having no effect on performance at all [Bartholomeusz et al., 2003; Roesch-Ely et al., 2005].

An alternative technique for modulating the dopaminergic system has been global depletion of DA, through acute tyrosine/phenylalanine depletion (TPD). Reducing the availability of the DA (and noradrenaline) precursors tyrosine and phenylalanine consequently reduces the synthesis and release of brain dopamine [McTavish et al., 1999a,b,c; Milner et al., 1986; Montgomery et al., 2003; Tam and Roth, 1997], with recent evidence that TPD reduces DA levels within the human striatum [Montgomery et al., 2003] and decreases amphetamine-induced DA release within the striatum [Leyton et al., 2004b]. Nevertheless, the effects of TPD on SWM performance have also been inconsistent. The first study to assess the effects of TPD on SWM revealed TPD-related deficits on two SWM tasks, a delayed-recognition task and a self-ordered strategic search task (impaired strategy was observed) [Harmer et al., 2001]. These findings were supported by Harrison et al. [2004], who observed impaired accuracy on a spatial (but not non-spatial) delayed-recognition task. However, two subsequent studies [McLean et al., 2004; Roiser et al., 2004] were unable to replicate impairments on the self-ordered strategic search task, while Lythe et al. [2005] failed to observed impairment on a delayed-response task following TPD. Similarly, we were unable to replicate a TPD-related impairment on the spatial delayed-recognition task used by Harrison et al. [2004] [Ellis et al., 2005]. In this latter study, another task designed to test SWM (n-back) was also unimpaired by TPD. Mehta et al. [2005] also failed to observe working memory deficits at a group level following TPD.

To date, most research has only assessed the effects of TPD on external, performance-based behavioural measures. A recent study demonstrated the advantages of combined neuroimaging and behavioural testing in examining TPD effects on working memory, by demonstrating that although TPD did not alter performance at a group level, there was a correlation between reduced striatal DA levels (as indexed by striatal [11C]-raclopride binding) and performance changes on the delayed-recall task [Mehta et al., 2005]. Specifically, performance worsened in participants with the greatest reduction in striatal DA levels, with virtually no change (and/or subtle improvement) in performance observed in participants with minimal DA depletion. However, the scope of the Mehta et al. [2005] study was limited to assessing DA changes within the striatum, and was unable to examine TPD effects on task-related activation within the PFC and associated working memory networks. The importance of DA transmission within the PFC during working memory task performance is well demonstrated in non-human primates [Goldman-Rakic et al., 1996]. In humans, bromocriptine (a D_2 receptor antagonist) was shown to only modulate the parietal cortex during working memory performance [Kimberg et al., 2001], but the psychomotor stimulants methylphenidate and amphetamine (indirect catecholamine agonists) have been associated with task-related reductions in activity within the PFC [Mattay et al., 2000; Mehta et al., 2000; Schweitzer et al., 2004] and posterior parietal cortex [Mehta et al., 2000], which are regions consistently activated by working memory tasks [for a review, see Wager and Smith, 2003].

The current study therefore aimed to examine the effects of TPD on rCBF within the PFC and associated SWM networks during performance of the n-back task [Gevins and Cutillo, 1993; McEvoy et al., 1998]. The spatial n-back was employed for the following three reasons: (1) it has a well-established neuroanatomical network, consistently activating the PFC (specifically the right DLPFC), posterior parietal cortex, and anterior cingulate gyrus [for a review, see Owen et al., 2005], (2) it can be employed with a parametric variation in memory load, which linearly relates to activation within the working memory network [e.g. Braver et al., 1997; Cohen et al., 1997] and (3) patients with schizophrenia show performance impairments on the spatial n-back task, which have been correlated with rCBF within the PFC [for a review, see Manoach, 2003] and PFC D_1 receptor availability [Abi-Dargham et al., 2002]. In addition to the interaction effect of TPD on task-related rCBF, we also aimed to examine the main effect of TPD across all task conditions and predicted predominantly striatal increases in rCBF in line with the effects of the DA receptor antagonist sulpiride [Mehta et al., 2003].

Given that differences in response preparation and execution demands have been proposed as a possible cause of discrepant behavioural findings between studies following DA manipulation [i.e. Ellis and Nathan, 2001; Luciana and Collins, 1997; Mehta et al., 2001, 2003], a second aim of this study was to examine the effects of TPD on tasks of SWM with differing response demands. We designed two delayed-response tasks, matched closely on most parameters, excluding response preparation and output. Specifically, the first task—the delayed-recognition task—minimised response preparation and output, while the second task—the delayed-recall task—allowed response preparation during the delay. These tasks were administered after completion of the PET scanning and in addition to the n-back task used in the scanner. Task-selective effects of TPD would inform on the nature of putative SWM impairments following TPD, and as such, create alternative hypotheses. For example, a general effect of TPD on the mnemonic aspects of SWM would predict impaired performance on all tasks. However, a greater influence of TPD on tasks requiring internal response preparation and directionally guided fine-motor movement would predict impairment on the delayed-recall task, whereas a greater influence of TPD on tasks requiring the "matching" of the internal stimulus to a new external stimulus, with less focus on response preparation and execution, would predict impairments on the delayed-recognition task.

METHOD

Participants

Eleven healthy right-handed male participants (mean age = 48.27 years, SD = 11.16; mean verbal IQ = 121.01, SD = 3.21; [NART: Nelson and Willison, 1991]) were recruited for the study through advertisements in the national press. All participants were thoroughly screened by a trained psychiatrist (V.M.), with history of psychiatric, neurological or chronic medical conditions (including cardiovascular illness), drug/mediation use or substance abuse comprising exclusion criteria. Ten of the 11 participants were non-smokers, with the one smoker abstaining from his normal 1 cigarette per day for 48 h prior to each day of testing. All participants gave written informed consent to participate in the study, which was approved by the Hammersmith, Queen Charlotte's and Chelsea and Acton Hospitals Research Ethics Committee and the Administration of Radioactive Substances Advisory Committee (UK).

The study followed a double-blind, placebo-controlled, repeated measures crossover design. Each participant completed two scanning sessions, separated by a minimum 5-day washout period. Six of the 11 participants received TPD in their first session. One participant failed to successfully complete the control task during the first scanning session, and hence was removed from PET analysis and accompanying n-back behavioural data analysis. Therefore, the PET component of the study includes 10 participants, and the post-scan behavioural testing (delayed-response tasks) includes 11 participants.

Procedure

Participants arrived at the laboratory at approximately 1030 hours on the morning of each testing session, having consumed a low protein diet (<25 g) in the preceding 24 h and having fasted from 1900 hours the previous evening. On arrival, the medical physician screened participants, and if deemed suitable for the study, an intravenous cannula was inserted in the antecubital vein in the left arm. At approximately 1200 hours (Time 0), participants were given an amino acid drink (balanced drink comprised isoleucine 15 g, leucine 22.5 g, lysine 17.5 g, methionine 5 g, valine 17.5 g, threonine 10 g, tryptophan 2.5 g, tyrosine 12.5 g and phenylalanine 12.5 g. TPD amino acid drink was identical to the balanced drink but lacked tyrosine and phenylalanine). Drinks were prepared within a few minutes of oral administration by suspending in water flavoured with blackcurrant. The order of drink administration for the two scans was counterbalanced. Table I summarises the time course of each testing session. This protocol is identical to our previous studies [Harmer et al., 2001; Mehta et al., 2005; Montgomery et al., 2003] that demonstrated marked and significant reductions in plasma tyrosine/phenylalanine levels relative to large neutral amino acid levels. Blood samples taken for assessment of amino acid levels were not analysed due to technical difficulties with sample processing. Acquisition of PET emission scans occurred +5 h after the amino acid mixture, to coincide with peak behavioural and physiological effects of the drink [Harmer et al., 2001; Harrison et al., 2004]. Post-scan behavioural testing (delayed-response tasks) took place outside the scanner at time +6 h. The testing day was complete at time +6.5 h and participants were provided with a high protein snack before they departed.

TABLE I. Summary of the time-course of testing sessions

Time	Procedure
1030 hours	Participant arrives at laboratory
1155 hours	Visual analogue scales administered
1200 hours (Time 0)	Amino acid drink administered
1300 hours (+1 h)	Standard task practice
1430 hours (+2.5 h)	Snack (raw carrot) provided to participant
1700 hours (+5 h)	Visual analogue scales re-administered Acquisition of PET images
1800 hours (+6 h)	Post-scan behavioural testing
1830 hours (+6.5 h)	Testing completed. High protein snack provided to participants before departure.

Subjective feelings and side effects were monitored during the testing sessions. Visual analogue scales [Bond and Lader, 1974] were administered at baseline (pre-drink) and at +5 h (post-drink/pre-scan). A carrot was also provided to subjects at +2.5 h post-drink to reduce hunger. Participants were provided with a standard practice session on all tasks (described below) at approximately +1 h on both testing days.

PET Scans

All participants were scanned on two separate occasions. $H_2{}^{15}O$ PET was selected as the imaging modality in this study as it allows for comparison of sessions in order to define the "main effect" of drug across the task conditions, in addition to providing excellent coverage of the brain. Each scanning session resulted in eight measures of rCBF, obtained using the Siemens/CTI ECAT EXACT 3D camera with the $H_2{}^{15}O$ bolus method [Raichle et al., 1983]. The camera has a full-width half-maximum resolution (FWHM) of 4.5 × 4.5 × 4.42 mm, and a 23.4 cm axial view of the field [Spinks et al., 2000]. Participants lay supine in the scanner with their head secured by a foam-lined fibreglass headholder, and strap over the forehead. Head position was monitored inter-scan, by comparing pen lines drawn on the nose and cheek with fixed laser guide lights, and participants were returned to original position between scans if required. For each scan, ~6 mCi $H_2{}^{15}O$ was infused in saline for 20 s at a rate of 10 ml/min, using the intravenous cannula in the left arm. A 30 s background frame was then acquired prior to the 90 s emission scan. There was an 8 min inter-scan interval to allow for decay of the radiotracer and set-up of the next cognitive task. A transmission scan was collected prior to emission scans to allow correction for attenuation effects, and scans were reconstructed using a ramp filter.

Cognitive Tasks

Spatial working memory n-back task

This task of SWM with a sustained attention component [Gevins and Cutillo, 1993; McEvoy et al., 1998] was run on a Toshiba computer and presented on a Illyana touch screen monitor, driven by a Microtouch™ touchscreen driver, suspended above the PET camera. The task involved the presentation of a series of white dots on a black background (with a central white fixation cross). Each dot was presented for 250 ms, with inter-stimulus intervals of 2,250 ms. Participants were required to indicate whether each dot was in the same location as the dot "n-back" (either 1- or 2-back), by pressing the "yes" or "no" button on the touch screen. The visuo-motor control task involved alternating responses between the "yes" and "no" buttons, regardless of the location of each dot.

For each scanning session, participants completed 3 × 1-back, 3 × 2-back and 2 × control tasks whilst rCBF was measured. Tasks were presented in a pseudorandom order that was fixed within subjects, but varied between subjects.

Post scan neuropsychological testing

Following scan acquisition, participants completed the three tasks whilst sitting upright in a semi-darkened room at arms length from a touch screen monitor. The two delayed-response tasks were completed first (counter-balanced across participants) followed by the reaction time task. The battery lasted ~20 min.

Both delayed-response tasks (recall and recognition) were run on a Dell computer connected to a Microtouch™ touchscreen and involved the presentation of black dots (displayed for 250 ms) on a white screen with a black central fixation cross. Performance changes following DA manipulation have been observed following an 8 s delay in recall tasks [Luciana and Collins, 1997; Luciana et al., 1992; Mehta et al., 2004], and performance changes in delayed recognition have been observed at delays of more than 8 s [Muller et al., 1998]. Therefore, both tasks included two inter-stimulus interval lengths; a "shorter" 4,000 ms delay, and a "longer" 12,000 ms delay.

For each delayed-response task, described in detail below, two blocks of trials were presented, each comprising 9 trials for each delay length (in pseudo-random order). Participants were instructed to focus on the central fixation cross throughout all trials and to try and remember the location of the dot during the delay.

Spatial working memory delayed-recall task

This task is an adaptation of the classical delayed-response task used in both experimental animal [Goldman-Rakic, 1999] and human research [Luciana et al., 1992; Mehta et al., 2004]. Three delay lengths were employed: "zero delay" control, 4,000 ms and 12,000 ms. A beep sounded at the completion of each delay, which signalled the participant to touch the screen as closely as possible to the position of the previous dot. Accuracy (distance from position touched to centre of target circle in millimeters) and response latency were recorded.

Spatial working memory delayed-recognition task

This task provides a measure of short-term recognition memory with minimal motor preparation and output de-

mands, and it is a modification of the delayed-response tasks used in human research [e.g. Harmer et al., 2001; Muller et al., 1998; Postle et al., 2003; Sahakian et al., 1988]. Two delay lengths were employed: 4,000 ms and 12,000 ms. A beep sounded at the completion of each delay, and two dots (probes) were presented in close proximity to each other. Participants were required to indicate which of the probe dots matched the original dot, by pressing one of two arrow buttons at the bottom of the screen.

Reaction time

This task was a modified version of the CANTAB reaction time task (CeNeS Ltd.) and was run on a Dell computer fitted with a Microtouch^{TM} touchscreen monitor. Participants rested their right hand on a pressure sensitive pad in front of a touch screen monitor. In the centre of the monitor there was an empty circle and participants were instructed that on the appearance of a yellow dot within this circle, they should attempt to touch this yellow dot as quickly as possible. An initial practice session was followed by two sets of 10 responses. Both reaction time (releasing the touchpad) and movement time (touchpad to screen) were recorded in milliseconds for all correct responses (i.e. touching within circle).

Subjective Feeling Assessment

Subjective feelings were obtained using a modified version of the Visual Analogue Scales [Bond and Lader, 1974] comprising 16 100-mm horizontal lines each representing a subjective feeling dimension, with opposing words at each end, e.g. happy – sad, alert – drowsy, amicable – antagonistic. The Visual Analogue Scales (VAS) were scored as two factors (alertness and tranquillity), consistent with the factor analysis of Herbert et al. [1976].

Statistical Analysis

All imaging data was analysed using SPM2 (www.fil.ion.ucl.ac.uk/spm) implemented in MATLAB version 5 (Mathworks, Sherborn, MA). Briefly, each scan was realigned using the mean image of the relevant session as reference, and stereotaxically normalised into a standard anatomical space developed at the Montreal Neurological Institute (MNI 152) by using a reference template provided in SPM2. Each image was smoothed using an isotropic Gaussian kernel (FWHM 12 mm) to compensate for gyral variability between participants and improve signal-to-noise ratio. All images were rescaled to a mean global CBF of 100 ml/100 g/min using ANCOVA. The condition, subject, and covariate effects were estimated according to the general linear model at each voxel [Friston et al., 1995]. Scan order was entered as a covariate of no interest to control for possible effects of time or order.

Appropriately weighted linear contrasts were used to identify changes in rCBF on a voxelwise basis [Friston et al., 1995]. The set of voxel values resulting from each contrast constitutes a statistical parametric map of the t statistic. As the working memory networks have previously been well defined within the literature [see Owen et al., 2005], linear contrasts of task related effects are reported at $P < 0.001$ uncorrected for multiple comparisons. In order to reduce the likelihood of Type 1 error, results of contrasts examining the main effect of treatment condition and interaction effects between treatment condition and task were thresholded at $P < 0.05$, corrected for multiple comparisons. Small volume correction (SVC) statistics [Worsley et al., 1996] were used to examine a priori predictions of effects within the striatum and the PFC. Specifically, the main and interaction effects of treatment condition within the striatum were examined as TPD-induced rCBF changes in this region were predicted [Mehta et al., 2003]. Further, interaction effects between treatment condition and task were examined within the PFC, with PFC defined as cortical regions anterior to the precentral gyrus using a maximum probability atlas [Hammers et al., 2003]. Simple linear regression was used to examine possible relationships between changes in task-related rCBF and corresponding change in cognitive performance (between placebo and TPD conditions). The cluster threshold was set to 5 for all figures. Peak voxels within significant clusters are reported using both MNI and Talairach co-ordinates [Talairach and Tournoux, 1988]. Talairach coordinates were estimated using MNI2TAL (www.mrc-cbu.cam.ac.uk/Imaging). Brodmann areas are associated with activated clusters where appropriate as estimated by Talairach and Tournoux [1988].

All behavioural data was analysed within SPSS (SPSS, Chicago, IL), using repeated measures analysis of variance (ANOVA). The standard error of the difference of the means (SED) (the index of variation commonly used in the crossover design) is reported for all data, as defined by Cochran and Cox [1957]: $\mathrm{SED} = \sqrt{2 \times \mathrm{MSe}/n}$, where MSe is the mean square error (or residual) term and n is the total number of observations made. Standard errors of the means are also reported in parenthesis following means.

RESULTS

Spatial N-Back Performance

The main measures of this task were accuracy (percentage correct) and latency, and are presented in Table II. As expected, participants were slower and made more errors with increasing load on the n-back task [significant linear fit for accuracy: $F(1,9) = 15.08$, $P < 0.01$ and latency: $F(1,9) = 36.30$, $P < 0.001$]. However, there was no significant interaction between amino acid mixture (TPD or placebo) and n-back level (control task, 1-, 2-back) for either latency [$F(1.95,17.58) = 0.19$, $P > 0.1$, with Greenhouse-Geisser correction] or percentage correct [$F(1.32,11.88) = 0.11$, $P > 0.1$, with Greenhouse-Geisser correction]. Further, there was no main effect of drug for either latency [$F(1,9) = 0.71$, $P > 0.1$] or percentage correct [$F(1,9) = 0.92$, $P > 0.1$].

TABLE II. Mean and standard error (SEM) values for behavioural data[a]

	Accuracy			Latency		
Task and delay	Placebo	TPD	SED	Placebo	TPD	SED
N-back task						
0-back	98.2 (0.6)	97.8 (1.1)	0.6	768.4 (93.2)	768.2 (99.9)	34.2
1-back	94.1 (1.9)	93.2 (1.7)	0.7	1278.7 (61.9)	1253.6 (67.7)	29.1
2-back	87.4 (2.9)	86.1 (2.5)	1.6	1383.5 (56.7)	1346.9 (63.3)	25.9
Recall						
0 s	8.2 (1.0)	9.4 (0.9)	0.4	1249.7 (83.9)	1313.2 (104.8)	32.8
4 s	9.8 (1.1)	11.8 (1.0)	0.7	1160.3 (60.7)	1185.4 (68.2)	44.2
12 s	12.3 (1.4)	13.2 (1.5)	1.0	1246.0 (57.0)	1200.6 (70.1)	39.5
Recognition						
4 s	90.9 (3.4)	89.4 (5.2)	2.7	2219.6 (75.6)	2204.7 (132.8)	77.6
12 s	88.9 (4.7)	92.4 (3.5)	1.5	2172.9 (73.1)	2224.8 (108.1)	70.3

[a] Given are n-back accuracy (percentage correct), delayed-recall accuracy (distance from target in millimetres), delayed-recognition accuracy (percentage correct), and latency (ms) for all tasks, following TPD and placebo.

The possible mediating effect of baseline working memory was examined by separating participants based on their median 2-back score (under placebo conditions) and entering this variable as a between participants factor. This analysis revealed no significant effects for any of the above measures (all $P > 0.1$).

Spatial Working Memory Delayed-Recall Performance

The main performance measures for this task are shown in Table II. As expected, there was a main effect of delay on accuracy [$F(2,20) = 15.91$, $P < 0.001$], with the distance from response to target location increasing with increased delay—supported by a significant linear fit contrast statistic [$F(1,10) = 21.2$, $P < 0.01$]. However, no interaction between amino acid mixture and delay condition (0, 4, 8 s) was observed for either accuracy [$F(2,20) = 0.60$, $P > 0.1$] or latency [$F(2,20) = 2.14$, $P > 0.1$]. Further, no significant main effect of drug was observed for either accuracy [$F(1,10) = 2.18$, $P > 0.1$] or latency [$F(1,10) = 0.10$, $P > 0.1$].

The baseline performance analysis revealed no interaction between baseline working memory (high or low, as defined above) and change in performance in either accuracy or latency (both $P > 0.05$).

Spatial Working Memory Delayed-Recognition Performance

The main performance measures for this task (percentage accuracy, and latency) are shown in Table II. There was no significant interaction between amino acid mixture and delay observed for either accuracy [$F(1,10) = 1.79$, $P > 0.1$] or latency [$F(1,10) = 1.60$, $P > 0.1$]. Further, no main effect of either drug (accuracy: [$F(1,10) = 0.16$, $P > 0.1$; latency: $F(1,10) = 0.03$, $P > 0.1$) or task difficulty (accuracy: $F(1,10) = 0.05$, $P > 0.1$; latency: $F(1,10) = 0.13$, $P > 0.1$) was observed.

The working memory baseline analysis revealed no significant effects on any measure (all $P > 0.1$).

Reaction Time/Movement Time Performance

The data for two participants were incomplete, and therefore analysis was performed on eight participants. One-way repeated measures analysis of variance was conducted for both reaction time and movement time measures. No significant difference was observed between amino acid drink conditions on either measure [reaction time: $F(1,7) = 0.42$, $P > 0.1$; movement time: $F(1,7) = 0.43$, $P > 0.1$]; see Table III for values.

Visual Analogue Scales

TPD did not significantly influence subjective feeling scores. There was no significant interaction between drink condition and time for either alertness [$F(2,20) = 0.95$, $P > 0.1$] or tranquillity [$F(2,20) = 1.6$, $P > 0.1$]. Further, there was no effect of the study day itself on subjective feelings, with no main effect of time on alertness [$F(2,20) = 0.39$, $P > 0.1$] or tranquillity [$F(2,20) = 0.21$, $P > 0.1$]; see Table IV for values.

Task-Related Activations

The effects of the SWM task on rCBF were examined in the placebo condition alone (to avoid confounding task activations with the effects TPD) using a multisubject condition and covariates PET model in SPM2. Glass brain images for the n-back task activations (2- plus 1-back, minus control task) are presented in Figure 1a, with details of the associated maxima presented in Table V. As expected, the task activated a distributed network including

TABLE III. Mean and standard error (SEM) scores for reaction time and movement time (ms) following TPD and placebo

Task	Placebo	TPD	SED
Reaction time	300.9 (13.6)	310.5 (20.3)	9.8
Movement time	411.7 (26.8)	436.7 (39.7)	24.0

TABLE IV. Mean and standard error (SEM) scores for the VAS factors (alertness and tranquillity)

	Placebo		TPD		
Task measure	Pre	Post	Pre	Post	SED
Alertness	73.3 (5.8)	74.1 (5.9)	75.9 (5.3)	72.7 (6.2)	1.7
Tranquillity	72.9 (2.5)	73.8 (3.4)	70.8 (2.8)	69.6 (3.6)	1.0

the right middle frontal gyrus (Brodmann area 9/46), the left supplementary motor cortex (BA 6), both superior and inferior regions of the parietal cortices, bilaterally (BA7/40), and the cingulate gyrus, in line with previous neuroimaging studies and a recent meta-analysis [Cohen et al., 1997; Jansma et al., 2000; Owen et al., 2005; Zurowski et al., 2002].

Memory Load Effects

Table V presents maxima of memory load effects, with glass brain images presented in Figure 1b. As expected, a number of regions within the working memory network (as defined using the task-related activation identified above) were sensitive to memory load. Load-related increases in activation were located within the PFC (BA 9/46, right hemisphere; BA 6, left hemisphere) and bilaterally within the parietal cortex (BA 7). Analysis of possible load effects over the whole brain revealed an additional and dominant load related effect in the rostral frontal cortex (BA 10/11).

Main Effect of TPD on rCBF

The main effects of TPD on rCBF were examined by averaging across all tasks (to avoid confounding the main effects of TPD with task specific activations). Table VI shows maxima of rCBF increases and decreases following TPD, with glass brain images presented in Figure 2. Small volume correction (SVC) analysis [based on the results of Mehta et al. 2003], using an MRI derived striatal region of interest map [Mawlawi et al., 2001], revealed TPD related increases in rCBF in the left putamen. Further examination across the whole brain showed signal increases in the hippocampal region, bilaterally (see Fig. 3), and the left inferior and superior frontal gyri. TPD-related reductions in rCBF were observed in the right inferior temporal gyrus, anterior cerebellar lobe (culmen) and pons.

Interaction Effects Between Treatment Condition and Task

TPD caused no augmentation or attenuation of the SWM-related activity within the PFC or striatum (using SVC analysis). Small volume correction analysis was also performed within the working memory network (using a

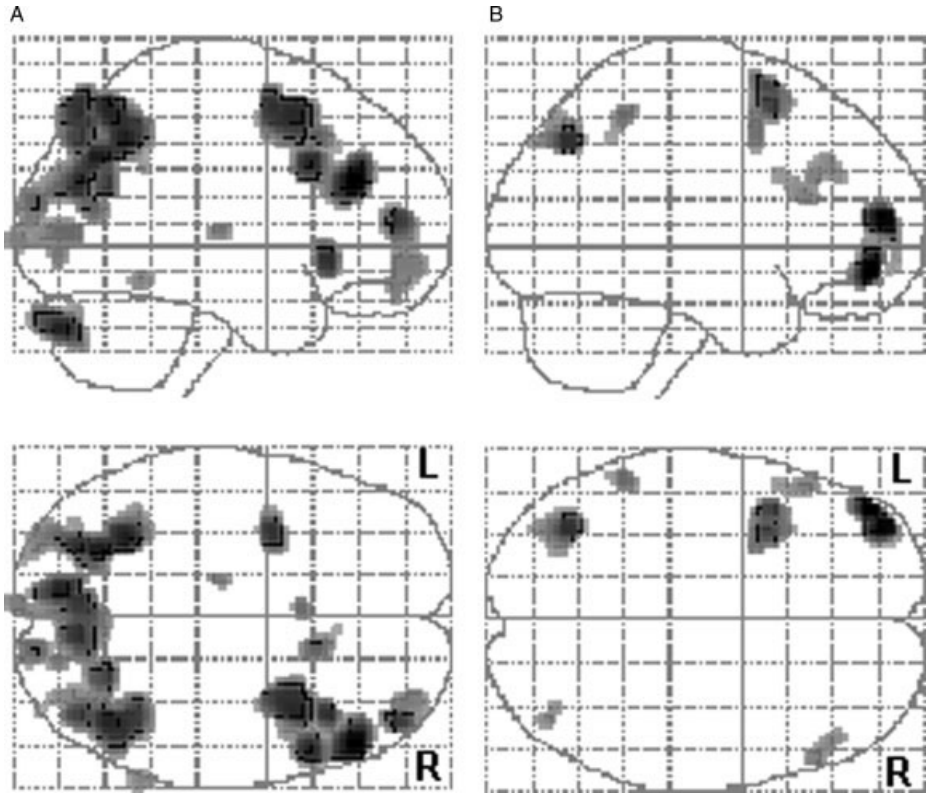

Figure 1.
Glass-brain activation maps for (**a**) the spatial n-back task (1- + 2-back) (**b**) memory load related increases between the 1-back and 2-back task. Both displayed at a threshold of $P < 0.001$ (uncorrected for multiple comparisons).

TABLE V. Peak increases in rCBF during the spatial n-back task (1- and 2-back tasks), and memory load related rCBF increases for the placebo condition scans

Region[a]	MNI coordinates	Talairach coordinates	Cluster size	Peak *t* value	*P* value
Task activation (1- and 2-back)					
R DLPFC (9/46)	50, 34, 26/52, 16, 32	50, 34, 22/ 52, 17, 29	1,425	5.36/4.63	$P < 0.001$[b]
L MFG (6)	−34, 2, 54	−34, 4, 50	240	4.51	$P < 0.001$
L inferior parietal lobe (7/40)[c]	−32, −56, 44	−32, −52, 43	912	4.81	$P < 0.001$
Superior parietal lobe (7)[d]	42, −62, 54	42, −58, 52	2,611	4.53	$P < 0.001$
Cingulate gyrus (32)	12, 20, 44	12, 21, 39	98	3.89	$P < 0.001$
Occipital lobe	14, −92, 18	14, −88, 21	186	3.72	$P < 0.001$
Memory load (2-back – 1-back)					
Anterior PFC (10/11)	−44, 50, −10/−36, 56, 12	−44, 48, −11/−36, 55, 8	387	4.87	$P < 0.001$[b]
L MFG (6)	−32, 6, 52	−32, 8, 47	23	3.48	$P < 0.001$
L inferior parietal lobe (7)	−36, −64, 44	−36, −60, 43	14	3.51	$P < 0.001$
R superior parietal lobe (7)	40, −76, 44	40, −72, 44	31	3.34	$P < 0.001$
R DLPFC (9/46)	56, 28, 26	55, 28, 22.5	64	3.47	$P < 0.001$

[a] Brodmann area given for regions in parenthesis.
[b] Denotes clusters that remain significant after correction for multiple comparisons across the whole brain.
[c] This cluster extends posteriorly into the occipital lobe with significant sub-peaks not tabulated.
[d] This cluster extends medially encompassing other significant subpeak not tabulated.

mask of working memory networks created from the task-related activation identified above) and no significant signal changes were detected. In addition, no significant signal changes were detected in areas outside the working memory network (even after exploring sub-threshold changes using a statistical threshold of $P < 0.001$ uncorrected for multiple comparisons across the whole brain).

Correlation Between Changes in Task-Related rCBF Activation and Cognitive Performance Following TPD

Possible relationships between changes in task-related rCBF and corresponding change in cognitive performance (between placebo and TPD conditions) were examined for both the 1-back and 2-back condition using simple linear regression. There were no significant correlations between rCBF changes and either performance latency and performance accuracy changes in either the 1-back or 2-back condition across the whole brain, or within a striatal region of interest (all $P > 0.05$).

DISCUSSION

This was the first study to examine the effects of TPD on rCBF in humans. We were able to demonstrate clear and marked changes in rCBF in both cortical and limbic regions following TPD, including changes within the striatum—in line with evidence that TPD decreases subcortical DA transmission in humans [Leyton et al., 2004b; Montgomery et al., 2003]. However, the main finding of this study is that despite these task-independent effects of TPD on cerebral blood flow, we were unable to see task-dependent modulations of rCBF following TPD using the n-back task. In addition, TPD did not influence performance on either the n-back task or two additional delayed-response tasks of SWM with varying response preparation and execution demands.

Task-Related Activation

The results of this study do not support TPD-induced modulations of task-related rCBF during the n-back task and appear to contrast evidence of a modulatory effect of

TABLE VI. Peak changes in rCBF following TPD

Region[a]	MNI coordinates	Talairach coordinates	Cluster size	Peak *t* value	*P* value
rCBF increases					
Left putamen	−28, −14,0/−30, −14, −4	−28, −14,1/−30, −14, −3	170	9.62/9.13	$P < 0.05$
L hippocampal region/	−24, −26, −6	−24, −25, −4	13,758	13.9/10.89	$P < 0.05$
R parahippocampal gyrus	16, −28, −12	−16, −28, −9			
L inferior frontal gyrus (44)	−54, 14, 10	−53, 14, 9	9,745	9.75	$P < 0.05$
rCBF decreases					
R inferior temporal gyrus (20)	42, −18, −34	42, −16, −28	2,333	10.4	$P < 0.05$
Left cerebellum/culmen	−28, −56, −36	−28, −56, −27	729	8.26	$P < 0.05$
Pons	−4, −22, −32	−4, −23, −26	274	7.58	$P < 0.05$

[a] Brodmann area given for regions in parenthesis.

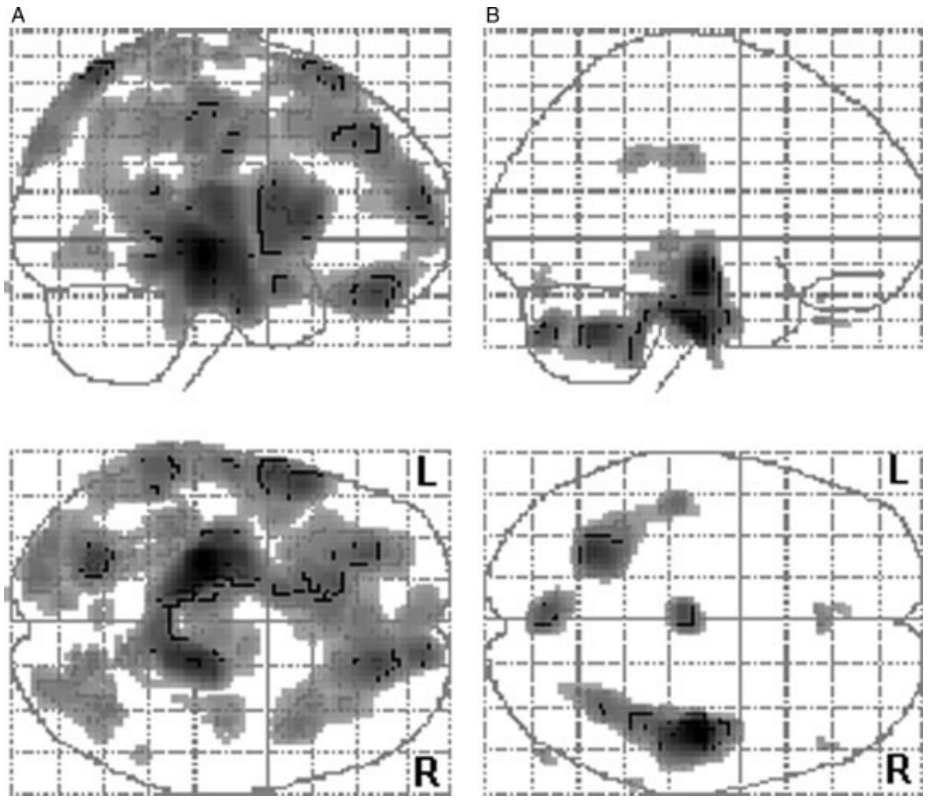

Figure 2.
Glass-brain activation maps for main effect of TPD on rCBF (**a**) increases following TPD (compared with placebo) (**b**) decreases following TPD. Both displayed at a threshold of $P < 0.05$ (corrected for multiple comparisons).

DA within the PFC during SWM tasks in non-human primates [for review, see Goldman-Rakic et al., 1996], and evidence in humans that increased DA receptor stimulation can modulate indices of working memory related neuronal activity in the parietal cortex [Kimberg et al., 2001]. The current results are also inconsistent with psychomotor stimulant (indirect catecholaminergic agonist) induced changes in task-related PFC activity [Mattay et al., 2000; Mehta et al., 2000; Schweitzer et al., 2004]. However, our findings add to the studies that have failed to demonstrate DA D_2 receptor agonist [Kimberg et al., 2001] and antagonist [Mehta et al., 2003] modulation of the PFC during working memory performance. Taken together with our findings, these studies highlight the difficulty of clearly elucidating the influence of dopaminergic modulation of working memory-related neural networks in humans that is clearly predicted on the basis of work in experimental animals. The lack of TPD-related modulation of SWM neu-

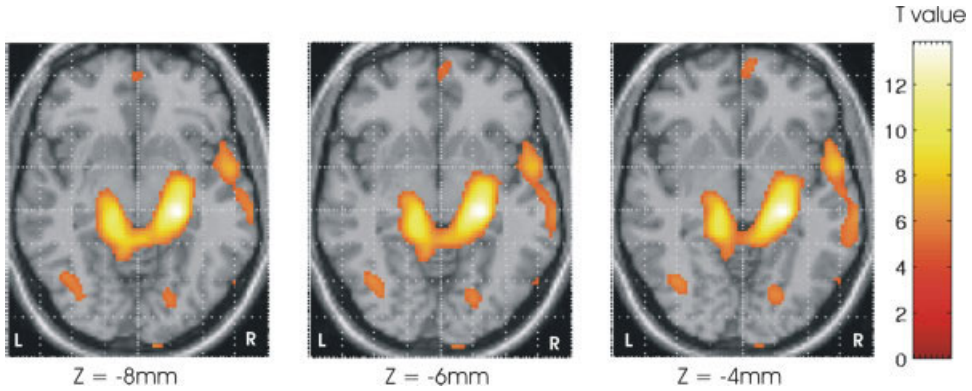

Figure 3.
Transverse sections (at $z = -8$ mm, -6 mm, -4 mm) showing TPD-related increases in the area of the parahippocampus. Coloured bar shows t values. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

ral networks was not the result of limited task-related activations, as this study again showed the spatial n-back task activates a robustly-defined network of brain areas including the PFC [Owen et al., 2005]. This network was sensitive to memory load, with increased memory load associated with increased DLPFC and posterior parietal cortex activation, consistent with previous findings [e.g. Braver et al., 1997; Cohen et al., 1997]. Additional memory load related increases were observed within the anterior PFC (BA 10), which fits well within a developing understanding of the role of the anterior PFC [for a review, see Ramnani and Owen, 2004], specifically in terms of a possible role in sub-goals within dual tasks [Koechlin et al., 1999] and in integration of sub-goals within working memory [Braver and Bongiolatti, 2002].

The lack of modulation of task-related rCBF was in the context of clear changes in rCBF following TPD suggesting that extent of DA depletion may have been insufficient to modulate SWM networks. TPD produces less DA depletion within the striatum than α-methylparatyrosine (AMPT), a more aggressive method of depleting DA and noradrenaline by inhibiting tyrosine hydroxylase [see Verhoeff et al., 2002, 2003]. Following TPD, changes in [11C]-raclopride binding (indexing striatal DA depletion) are around 6% [Montgomery et al., 2003]. In contrast, following AMPT (in the non-human primate), changes in SPECT radiotracer [123I]-IBZM binding are ∼25% [Laruelle et al., 1997]. The limited amount of DA depletion observed following TPD is despite evidence demonstrating TPD to be a highly reliable and effective method of depleting peripheral plasma levels of tyrosine and phenylalanine. All studies within the literature report robust and highly significant depletion of tyrosine and phenylalanine levels (or the ratio between tyrosine/phenylalanine and other large neutral amino acids) in the TPD compared with those in the balanced condition [Harrison et al., 2004; Leyton et al., 2000, 2004a,b; Lythe et al., 2005; McLean et al., 2004; McTavish et al., 2004, 2005; Mehta et al., 2005; Roiser et al., 2004, 2005]. Plasma levels of tyrosine and phenylalanine were not available in the current study (see Methods); however this study was conducted in the same laboratory in which the Mehta et al. study [2005] was conducted (which produced significant tyrosine depletion over 90%, consistent with the TPD literature), using the same procedures, amino acid stock and measuring scales. While it is suggested that TPD is a reliable method of depleting plasma tyrosine and phenylalanine levels, this may not result in substantial or significant *dopamine depletion* in all participants.

Indeed, recent evidence suggests that plasma levels of tyrosine and phenylalanine are not good predictors of cognitive function [Mehta et al., 2005]. In contrast, however, Mehta et al. [2005] recently demonstrated, using [11C]-raclopride PET, that only participants with a high level of DA depletion showed impaired performance following TPD. In the current study, there was no evidence of a similar relationship between task-related rCBF changes and performance changes following TPD across the whole brain. Thus, while changes in central DA levels may predict changes in performance, there are no brain regions where rCBF changes confer a similar predictive value. The reasons for this are currently unclear, although it may relate to the lower selectivity of rCBF measures compared with [11C]-raclopride imaging for dopamine-related changes, or a poor temporal specificity for potential effects that may be present within discrete subcomponents of the task.

Behavioural Effects

In the current study, we examined whether differences in task demands may be a source of discrepant behavioural findings [for discussion, see Luciana and Collins, 1997; for discussion, see Mehta et al. 2001, 2003], and our findings suggest that this is unlikely, at least for DA depletion following TPD. We did not observe an effect of TPD on two versions of the delayed-response task (recall and recognition) differing in the ability of subjects to prepare a response during the delay period, as well as the n-back task which is a complex task involving information manipulation and updating, and sustained attention. While our findings are at odds with earlier studies showing delayed recognition impairments after TPD [Harmer et al., 2001; Harrison et al., 2004], they are consistent with a number of more recent findings which suggest that, overall, TPD does not have a measurable effect on working memory performance measures [Ellis et al., 2005; Lythe et al., 2005; McLean et al., 2004; Mehta et al., 2005; Roiser et al., 2004]. It is unlikely that the lack of behavioural effect across all tasks in this study was due to insensitivity of these tasks to dopaminergic manipulations, as they are arguably the most commonly used SWM task types, and performance has been previously linked to dopaminergic levels and/or modulation in all three tasks [i.e. Luciana and Collins, 1997; Luciana et al., 1992; Mehta et al., 2004; Meyer-Lindenberg et al., 2001; Muller et al., 1998; Park and Holzman, 1992]. While the lack of behavioural effect may be due to under-powering of the study, the current sample size ($N = 11$) is comparable with that of both Harmer et al. [2001] ($N = 12$) and Harrison et al. [2004] ($N = 13$), who observed TPD-related performance effects. Furthermore, using tasks identical to those of Harrison et al. [2004] and Harmer et al. [2001], two larger studies by Ellis et al. [2005] ($N = 18$) and McLean et al. [2004] ($N = 40$, between subjects design) failed to replicate TPD-related performance effects. Indeed, power analysis of our findings suggest that for the changes in performance accuracy of the 1- and 2-back task to be significant, sample sizes of at least 94 and 205 respectively would have been necessary, and similarly sample sizes of at least 50 would be required to detect significant performance accuracy changes in either the 4- or 12-s versions of the delayed-recall and delayed-recognition tasks, questioning the significance of such small effect sizes. Therefore, the lack of effects of TPD on performance of three working memory tasks appears to be in line with the

overall lack of effect on task-related rCBF across the entire brain during performance of the n-back task.

Main Effect of TPD on rCBF

TPD induced widespread increases in rCBF, with maximal increases in the region of the parahippocampal gyrus (bilaterally) and the left inferior frontal gyrus. While the mechanisms responsible for these changes are unclear, they are likely to comprise a combination of effects that include, though are not exclusively, dopamine-related effects. Firstly, rCBF or blood oxygen dependent (BOLD) signal changes following pharmacological manipulation rely on the assumption that coupling between blood flow and neuronal activity remains relatively constant across all brain regions. Despite dopaminergic system being the most extensively studied on this matter and evidence suggesting no significant neurovascular (NV) uncoupling following DA modulation [e.g. Arthurs and Boniface, 2002; McCulloch, 1982; McCulloch et al., 1982], the effects of TPD on NV coupling are unknown. Large neutral amino acids (LNAA) methionine, arginine and homocysteine (which is converted to cysteine) can alter peripheral vasculature reactivity [Bellamy et al., 1998; Chambers et al., 2001; Frame, 1999; Rosengarten et al., 2003; Usui et al., 1999] and although cerebral vasculature has greater compensatory range than peripheral vasculature in minimising vascular decoupling [Rosengarten et al., 2003], TPD results in increased levels of LNAA and it remains possible that NV uncoupling contributed to the observed main effects of TPD on measured rCBF.

Notwithstanding these considerations, the pattern of TPD-related rCBF increases observed in the current study overlap with increases in glucose metabolism observed following AMPT (in 7 participants who did not experience relapse of depression) [Bremner et al., 2003]. We suggest these increases (in rCBF or glucose metabolism) may be partly due to compensatory mechanisms. In rodents, loss of striatal dopaminergic terminals is accompanied by apparent increases in DA synthesis and release from remaining DA terminals, and increased activity of the rate-limiting enzyme tyrosine hydroxylase (TH) [Zigmond et al., 1984]. Similarly, loss of noradrenergic terminals can results in increased TH activity specifically within the hippocampus, as a compensatory mechanism to maintain catecholaminergic influence on target cells within the brain [Acheson and Zigmond, 1981; Acheson et al., 1980]. Although these manipulations are more severe than that performed in our study, the data fit well with the our findings of maximal rCBF increases in the hippocampal region, possibly reflecting similar compensatory increases in TH in order to promote dopamine/noradrenaline synthesis and release under TPD conditions. TPD may also influence interacting neurotransmitter systems. For example, anatomical and pharmacological data suggest an apparent opponent partnership between DA and serotonin [e.g. Azmitia and Segal, 1978; Fletcher, 1991; Kapur and Remington, 1996]. Further, infusion of the serotonin precursor L-tryptophan reduces the ratio of tyrosine to large neutral amino acids [Heuther et al., 1992], biochemical markers of DA synthesis [Hashiguti et al., 1993], and "dopaminergic" behavioural responses (i.e. increased locomotor activity) [Molina et al., 2001]. It is therefore possible that DA depletion may similarly initiate increases in serotonergic transmission. Overall, we suggest the widespread rCBF increases following TPD demonstrates that globally modulating tyrosine/phenylalanine levels has a complex effect on neurophysiology, which is likely to reflect not only changes in DA levels but compensatory actions of the catecholaminergic systems, and interactions with other neurotransmitter systems.

CONCLUSIONS

These findings question the capacity of TPD to adequately modulate dopamine function in order to influence SWM performance and associated neural networks. Despite clear task-related activations, and considerable changes in cerebral blood flow following TPD, there was no evidence of TPD-related modulation of task-related activations. Further, the lack of a measurable effect of TPD on spatial delayed-recall, delayed-recognition and n-back performance suggests that it is unlikely that task differences underlie the mixture of effects observed in previous studies to date. The implication of these findings, when taken together with previous studies, is that degree of dopaminergic depletion achieved by TPD in brain regions critical to working memory function (such as the parietal and frontal cortices) may be insufficient to consistently and robustly modulate SWM networks in humans.

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